

Applicant : Heather A. Boux et al.
Serial No. : 09/733,179
Filed : December 7, 2000
Page : 7

Attorney's Docket No.: 12071-006001

REMARKS

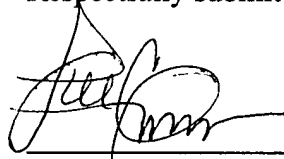
The present amendment is made to insert the Sequence Listing filed herewith into the application and to amend the specification and claims to include appropriate references to the SEQ ID NOs assigned to each sequence. No new matter has been added

Attached is a marked-up version of the changes being made by the current amendment.

Applicants ask that all claims be examined. Enclosed is a Petition for Extension of Time and a check for the required fee. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: December 10, 2001



Lee Crews, Ph.D.
Reg. No. 43,567

Fish & Richardson P.C.
225 Franklin Street
Boston, Massachusetts 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906

Version with markings to show changes made

In the specification:

The paragraph beginning at page 4, line 3, has been amended as follows.

The invention also features peptides that correspond to, or are antigenically equivalent to, regions of the Hsp70B' protein. The peptide can consist of five or more (*e.g.*, 5, 6, 7, 8, 10, or 12) consecutive amino acids from Hsp70B' protein (beginning at the second, fourth, sixth, or eighth residue), such as the following amino acid sequences:

(1) VPGGSSCGTQARQGDPSTGPI [(SEQ ID NO: __)] (SEQ ID NO:1) (*e.g.*, CGTQARQGDPSTGPI [(SEQ ID NO: __)] (SEQ ID NO:2 and CGTQARQGDPST [(SEQ ID NO: __)] (SEQ ID NO:3)); (2) RDKIPEEDRRKMQDKC [(SEQ ID NO: __)] (SEQ ID NO: 4) (*e.g.*, RDKIPEEDRRKMQ [(SEQ ID NO: __)] (SEQ ID NO:5); when these peptides are linked to keyhole limpet hemocyanin (KLH), they can include cysteine residues); (3) AHVFHVKGSLQEESLRDKIPEEDRRKMQ [(SEQ ID NO: __)] (SEQ ID NO:6) (*e.g.*, AHVFHVKGSLQEES [(SEQ ID NO: __)] (SEQ ID NO:7); (4) MQAPRELAVGID [(SEQ ID NO: __)] (SEQ ID NO:8), which is located in the N-terminal of Hsp70B' and, when linked to KLH includes a cysteine residue (*i.e.*, MQAPRELAVGID(C) [(SEQ ID NO: __)] (SEQ ID NO.:9)); (5) GSLQEESLRDKIPEE [(SEQ ID NO: __)] (SEQ ID NO:10).

The paragraph beginning at page 5, line 24, has been amended as follows.

Fig. 2 is a representation of the human Hsp70B' amino acid sequence [(SEQ ID NO: __)] (SEQ ID NO:11).

The paragraph beginning at page 5, line 23, has been amended as follows.

The invention features immunogenic peptides whose sequence is present in the Hsp70B' protein or whose sequence varies from the sequence of the Hsp70B' protein in such a limited way as to remain an antigenic equivalent of the naturally occurring peptide. For example, an Hsp70B' protein or peptide that contains one or more amino acid substitutions (*e.g.* one or more conservative amino acid substitutions) can be antigenically equivalent to the naturally occurring

Hsp70B' protein or peptide fragments thereof. Proteins and peptides that, upon administration to an animal, elicit the production of antibodies that specifically bind to Hsp70B' protein include the following: (1) VPGGSSCGTQARQGDGPSTGPI [(SEQ ID NO: __)] (SEQ ID NO:1) (e.g., CGTQARQGDGPSTGPI [(SEQ ID NO: __)] (SEQ ID NO:2) and CGTQARQGDGPST [(SEQ ID NO: __)] (SEQ ID NO: 3)); (2) RDKIPEEDRRKMQDKC [(SEQ ID NO: __)] (SEQ ID NO:4) (e.g., RDKIPEEDRRKMQ [(SEQ ID NO: __)] (SEQ ID NO:5); when these peptides are linked to keyhole limpet hemocyanin (KLH), they can include N-terminal cysteine residues); (3) AHVFHVKGSLQEESLRDKIPEEDRRKMQ [(SEQ ID NO: __)] (SEQ ID NO:6) (e.g., AHVFHVKGSLQEES [(SEQ ID NO: __)] (SEQ ID NO:7); (4) MQAPRELAVGID [(SEQ ID NO: __)] (SEQ ID NO:8), which is located in the N-terminal of Hsp70B' and, when linked to KLH includes a C-terminal cysteine residue (i.e., MQAPRELAVGID(C) [(SEQ ID NO: __)] (SEQ ID NO:9)); (5) GSLQEESLRDKIPEE [(SEQ ID NO: __)] (SEQ ID NO:10); and the Hsp70B' protein [(SEQ ID NO: __)] (SEQ ID NO:11).

The paragraph beginning at page 16, line 22, has been amended as follows.

Methods: Hsp70B' Antibodies were produced as follows. Hsp70B' antibodies were produced in rabbits, goats and mice with either synthetic peptides or recombinant Hsp70B' protein as immunogen. Eight peptides were chosen from the human Hsp70B' amino acid sequence. One of the Hsp70B' peptides, the NT peptide MQAPRELAVGID(C) [(SEQ ID NO: __)] (SEQ ID NO:9) corresponded to an N-terminal fragment. The other seven fragments were derived from the C-terminal half of the Hsp70B' protein and included the CC peptide (AHVFHVKGSLQEES; [SEQ ID NO: __] (SEQ ID NO:7), the CA peptide (RDKIPEEDRRKMQ; [SEQ ID NO: __] (SEQ ID NO:5), the CD peptide (RDKIPEEDRRKMQDKC; [SEQ ID NO: __] (SEQ ID NO:4); the CB peptide (CGTQARQGDGPSTGPI; [SEQ ID NO: __] (SEQ ID NO:2), the ECB peptide (VPGGSSCGTQARQGDGPSTGPI; [SEQ ID NO: __] (SEQ ID NO:1), the TCB peptide (CGTQARQGDGPST; [SEQ ID NO: __] (SEQ ID NO:3), and the CE peptide (GSLQEESLRDKIPEE; [SEQ ID NO: __] (SEQ ID NO:10). The CB peptide was also resynthesized on a separate occasion and designated CB2. All peptides were chemically coupled to KLH and animals were immunized with the peptide conjugates. Recombinant human

Hsp70B' protein was purified to ~90% homogeneity and was also used as an immunogen. Primary immunizations were administered in Freund's complete adjuvant and subsequent boosts were made in Freund's incomplete adjuvant. Animals were immunized and boosted on a monthly basis. Sera were collected at various time points and the antibody response to the immunizing protein or peptide was evaluated in an indirect enzyme immunoassay (EIA). Titres were established as the dilution factor at which the absorbance in the test sample was equal to 0.2 optical density units. In some instances, high-titre antisera from each set of animals were pooled and the antigen-specific antibody purified on peptide immunoaffinity columns.

The paragraph beginning at page 23, line 10, has been amended as follows.

Cloning and Expression of Recombinant Human Hsp70B'. Human Hsp70B' was cloned from heat shocked HeLa cells and expressed recombinantly in *E. coli*. Briefly, 2×10^7 HeLa cells were heat shocked for 2 hours at 44°C and then immediately harvested. Poly (A+) RNA was isolated from the heat shocked HeLa cells with a mRNA isolation kit (Boehringer Mannheim) and used to synthesize human Hsp70B' cDNA by RT-PCR. The 51 µl RT-PCR reaction mixture consisted of 1 µl of 10 mM dNTP (Perkin Elmer), 2.5 µl of 100 mM DTT (Boehringer Mannheim), 0.25 µl of 40 units/µl RNase inhibitor (Boehringer Mannheim), 10 µl of 5X RT-PCR buffer containing 7.5 mM MgCl₂ and DMSO (Boehringer Mannheim), 1 µl of enzyme mix (Boehringer Mannheim) containing Expand High Fidelity enzyme mix and AMV reverse transcriptase, 0.87 µg of poly (A+) RNA from heat shocked HeLa cells, and 1 µg each of: primer 1: 5'-GAAGCTTCACATATGCAGGCCCCACGGGAGCTCG-3' [(SEQ ID NO:)] (SEQ ID NO:12) and primer 2: 5'-GAAGCTCGAGTCAATCAACCTCCTCAATGA-3' [(SEQ ID NO:)] (SEQ ID NO:13).

The paragraph beginning at page 24, line 15, has been amended as follows.

Cloning and Expression of Recombinant His₆-Human Hsp70B (Fragment). The 741bp fragment that encodes a portion of the amino terminus region of the human Hsp70B was obtained from SPD-925, a human Hsp70B stress gene probe (Stressgen Biotechnologies). SPD-925 is supplied as a plasmid containing 3.15 kb of the 5' non-transcribed Hsp70B gene sequence,

CGTQARQGDPSTGPI [(SEQ ID NO: __)] (SEQ ID NO:2);
CGTQARQGDPST [(SEQ ID NO: __)] (SEQ ID NO:3);
RDKIPEEDRRKMQ [(SEQ ID NO: __)] (SEQ ID NO:5); and
GSLQEESLRDKIPEE [(SEQ ID NO: __)] (SEQ ID NO:10).

22. An antibody that specifically binds an Hsp70B' peptide consisting of five or more consecutive amino acid residues within one of the following amino acid sequences:

VPGGSSCGTQARQGDPSTGPI [(SEQ ID NO: __)] (SEQ ID NO:1);
RDKIPEEDRRKMQDKC [(SEQ ID NO: __)] (SEQ ID NO:4);
AHVFHVKGSLQEESLRDKIPEEDRRKMQ [(SEQ ID NO: __)] (SEQ ID NO:6); or
MQAPRELAVGID [(SEQ ID NO: __)] (SEQ ID NO:9).

24. An antibody that specifically binds a peptide that consists of one of the following amino acid sequences:

VPGGSSCGTQARQGDPSTGPI [(SEQ ID NO: __)] (SEQ ID NO:1);
RDKIPEEDRRKMQDKC [(SEQ ID NO: __)] (SEQ ID NO:4);
AHVFHVKGSLQEESLRDKIPEEDRRKMQ [(SEQ ID NO: __)] (SEQ ID NO:6); or
MQAPRELAVGID [(SEQ ID NO: __)] (SEQ ID NO:9);
AHVFHVKGSLQEES [(SEQ ID NO: __)] (SEQ ID NO:7);
CGTQARQGDPSTGPI [(SEQ ID NO: __)] (SEQ ID NO:2);
CGTQARQGDPST [(SEQ ID NO: __)] (SEQ ID NO:3);
RDKIPEEDRRKMQ [(SEQ ID NO: __)] (SEQ ID NO:5); and
GSLQEESLRDKIPEE [(SEQ ID NO: __)] (SEQ ID NO:10).

26. A kit for analyzing the expression of Hsp70B', the kit comprising an antibody that specifically binds an Hsp70B' peptide consisting of five or more consecutive amino acid residues within one of the following amino acid sequences:

VPGGSSCGTQARQGDPSTGPI [(SEQ ID NO: __)] (SEQ ID NO:1);
RDKIPEEDRRKMQDKC [(SEQ ID NO: __)] (SEQ ID NO:4);

AHVFHVKGSLQEESLRDKIPEEDRRKMQ [(SEQ ID NO: __)] (SEQ ID NO:6); or
MQAPRELAVGID [(SEQ ID NO: __)] (SEQ ID NO:9).

28. A method of obtaining an antibody that specifically binds Hsp70B', the method comprising administering to an animal a peptide consisting of five or more consecutive amino acid residues within one of the following amino acid sequences:

VPGGSSCGTQARQGDPSTGPI [(SEQ ID NO: __)] (SEQ ID NO:1);
RDKIPEEDRRKMQDKC [(SEQ ID NO: __)] (SEQ ID NO:4);
AHVFHVKGSLQEESLRDKIPEEDRRKMQ [(SEQ ID NO: __)] (SEQ ID NO:6); or
MQAPRELAVGID [(SEQ ID NO: __)] (SEQ ID NO:8).

29. The method of claim 28, wherein the peptide consists of one of the following amino acid sequences:

VPGGSSCGTQARQGDPSTGPI [(SEQ ID NO: __)] (SEQ ID NO:1);
RDKIPEEDRRKMQDKC [(SEQ ID NO: __)] (SEQ ID NO:4);
AHVFHVKGSLQEESLRDKIPEEDRRKMQ [(SEQ ID NO: __)] (SEQ ID NO:6); or
MQAPRELAVGID [(SEQ ID NO: __)] (SEQ ID NO:8);
AHVFHVKGSLQEES [(SEQ ID NO: __)] (SEQ ID NO:7);
CGTQARQGDPSTGPI [(SEQ ID NO: __)] (SEQ ID NO:2);
CGTQARQGDPST [(SEQ ID NO: __)] (SEQ ID NO:3);
RDKIPEEDRRKMQ [(SEQ ID NO: __)] (SEQ ID NO:5); and
GSLQEESLRDKIPEE [(SEQ ID NO: __)] (SEQ ID NO:10).